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Interaction between Chemical Mutagens with a Delayed Effect and Metabolites of Seeds

Communication I: The Cytogenetic Action of Mutagens as Related to the Physiological Condition of Seeds

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Summary. A study was made of the cytogenetic effect of mutagens with a delayed effect (ethylenimine and ethyl methanesulphonate) on *Crepis capillaris* seeds. The effect was found to depend on the physiological condition of the seeds. In seeds not subjected to prolonged storage, where only chromatid aberrations were occurring spontaneously, mutagens also induced chromatid aberrations only. If, however, because of physiological changes in the seeds (e.g. upon prolonged storage or when seeds were kept at an elevated temperature and humidity) a large number of chromosome-type aberrations appeared, they also appeared when the seeds were acted upon by mutagens with a delayed effect. The action of such mutagens was also found to depend on spontaneous mutation in seeds with different rates of germination. Special experiments showed that the interaction of ethylenimine with the metabolites of seeds in vitro leads to the formation of secondary active mutagens differing from ethylenimine in the nature of their action. The induction of chromosome-type aberrations by treating seeds with alkylating compounds may be due to the action of secondary mutagens.

Introduction

It is known that mutagens with a delayed effect on plant seedlings result in chromatid aberrations only. However, in some experiments, when seeds were treated with such mutagens, only chromatid aberrations were recorded (Moutschen-Dahmen and Moutschen-Dahmen, 1963, Sidorov, Sokolov, and Andreyev, 1965; Grinikh, 1969), while in others both chromatid and chromosome aberrations were observed (Moutschen and Degraeve, 1965; Ramanna and Natarajan, 1965; Gilot, Moutschen-Dahmen and Moutschen-Dahmen, 1967; Dubinina and Dubinin, 1968). It is important to find the causes for the appearance of chromosome rearrangements in seeds, since it is still not clear whether mutagens with a delayed effect induce aberrations only during chromosome replication (Froese-Gertzen, Konzak, Nilan and Heiner, 1964; Sidorov, Sokolov, and Andreyev, 1965; Andreyev, Sidorov and Sokolov, 1966) or whether they do so at every stage of the cell cycle (Auerbach 1945; Kimball, 1965; Dubinina and Dubinin, 1968).

It has been suggested that the cell may be damaged not only through direct interaction of the mutagen with the chromosomes, but also through the action of secondary mutagenic products (Sidorov, Sokolov and Andreyev, 1966). It cannot be ruled out that chromosome aberrations in seeds may be caused by reaction products of the mutagen with cell metabolites whose composition depends on the physiological condition of the seeds. Our experiments were designed to check this assumption.

Material and Methods

Dormant *Crepis capillaris* seeds and seedlings were treated with ethylenimine in concentrations of $2.3 \cdot 10^{-2}$ M (treatment period 1 hour) and $4.6 \cdot 10^{-2}$ M (for 3 hours), and with ethyl methanesulphonate (EMS) at 0.1 M concentration for 5—7 hours. The mutagen solutions were prepared in a phosphate buffer at pH = 7. They were used to treat seeds stored under room conditions from two months to six years, and seeds kept for 12 days at 53 °C and elevated humidity. After treatment, the seeds were rinsed in running water for 30 minutes and allowed to germinate in a 0.01% colchicine solution. The emerging seedlings were fixed in alcohol-acetic (3:1) and squashed acetocarmine preparations were made. The chromosome aberrations observed in the metaphase of diploid cells served as an indicator of the damaging effect of treatment.

A special in vitro study was also carried out in which ethylenimine was made to interact with substances separated from seeds. Ground *C. capillaris* seeds stored for different time periods were flooded with a mutagen solution of $2.3 \cdot 10^{-2}$ M (in quantities of equal volume). This mixture was used after 30 min. to treat seedlings. In the control, a phosphate buffer was added to the ground mass of seeds. After rinsing for 30 min. the seedlings were placed in a 0.01% colchicine solution and fixed at 4, 9, 15 or 20 hours later.

Results and Discussion

When spontaneous mutation was examined in seeds two months after harvesting, only 0.2% of chromatid aberrations were recorded (Tables 1 and 2). With increasing storage time, the number of aberrations in the seeds increased and the spectrum of aberrations changed. Whereas all the aberrations found in the seeds collected two months before were

Table 1. *Effect of ethylenimine on Crepis capillaris seeds stored for different time periods* (Ethylenimine concentration $2.3 \cdot 10^{-2}$M, treatment period 1 hour)

Storage time	2 months		6 months		1½ years		2½ years		3½ years		4½ years		6½ years	
Chromosome aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations
Chromatid, aberrations	300	11.6 ± 0.6	257	9.2 ± 0.6	450	12.0 ± 0.5	519	9.8 ± 0.4	224	5.4 ± 0.4	129	1.4 ± 0.2	111	3.4 ± 0.4
Chromosome aberrations	0	0	9	0	20	0.1 ± 0.5	113	1.1 ± 0.1	183	3.1 ± 0.3	325	5.1 ± 0.3	170	4.8 ± 0.4
Total number of aberrations	300	11.6 ± 0.6	266	9.2 ± 0.6	470	12.1 ± 0.5	632	10.9 ± 0.5	407	8.5 ± 0.4	454	6.5 ± 0.4	281	8.2 ± 0.2
Total number of metaphases examined	570		2742		3686		4964		3935		4410		2571	
Controls														
Chromatid aberrations	2	0.2 ± 0.1	6	0.2 ± 0.1	10	0.2 ± 0.11	22	0.6 ± 0.1	3	0.3 ± 0.1	60	1.5 ± 0.2	20	0.9 ± 0.2
Chromosome aberrations	0	0	9	0.3 ± 0.1	18	0.4 ± 0.1	38	1.1 ± 0.2	40	1.5 ± 0.2	98	2.2 ± 0.2	42	1.8 ± 0.3
Total number of aberrations	2	0.2 ± 0.1	15	0.5 ± 0.1	28	0.6 ± 0.1	60	1.7 ± 0.2	48	1.8 ± 0.3	158	3.7 ± 0.3	62	2.7 ± 0.3
Total number of metaphases examined	987		2916		3634		3399		2728		4300		2222	

all of the chromatid type, after six months of storage chromosome aberrations also appeared, in a progressively rising proportion. It was found that the spectrum of aberrations induced by ethylenimine and EMS (analyzing seedlings which emerged 48 hours after treatment) was also determined by the initial condition of the seeds (Tables 1 and 2). When no chromosome aberrations were spontaneously formed in seeds, they were also absent when mutagens with a delayed effect were used. However, when an appreciable number of chromosome aberrations occurred in the seeds through physiological changes caused by storage, these aberrations also appeared after treatment with mutagens.

The ability of mutagens with a delayed effect to induce chromosome aberrations was also revealed in seeds kept at an elevated temperature and humidity. Following such storage, 5.4% of aberrations, mainly of the chromosome type, were recorded in the seedlings (Table 3); ethylenimine induced 27.6% of aberrations in these seeds, 25% being of the chromosome type.

The dependence of the aberration spectrum produced by ethylenimine treatment on the physiological condition of the seeds was also observed in the experiments using seeds stored for two and seven months and taking into account the rate of germination of the seeds. It was found (Table 4) that in early-germinating seeds (up to 50 hours since the beginning of moistening) the spontaneous level of mutation was very low, but in seeds germinating later (in five to ten days) the percentage of aberrations was higher and included chromosome as well as chromatid aberrations. After treatment with ethylenimine, chromosome aberrations were found only in late-germinating seeds (Table 4). Thus seedlings that appeared 200 to 240 hours after ethylenimine treatment showed 2.2% of chromosome aberrations when from seeds stored for two months after harvesting, and 15.1% aberrations from seeds stored for seven months. As in the preceding experiments, the mutagen effect was a function of the physiological state of the seeds.

It cannot be excluded that during storage some specific metabolic products appear and accumulate in the seeds. The cytogenetic action of mutagens appears to depend not only on their chemical nature but also on their interaction with the cellular substances. Because of the great reactivity of alkylating compounds, we assume that these products react with the added mutagens to form secondary mutagens with a different type of action.

To check the validity of this assumption, special experiments were carried out to try to obtain in vitro active secondary mutagens through interaction between ethylenimine and metabolites isolated from seeds after different periods of storage, and also to ascertain the specificity of the mutagenic effect of the products obtained. If the assumption regarding

Table 2. *Effect of EMS on Crepis capillaris seeds stored for different time periods*

Storage time	2 months (concentration 0.1 M; time 5 hours)		6 months (concentration 0.1 M; time 7 hours)		$4^1/_2$ years (concentration 0.1 M; time 5 hours)	
Chromosome aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting spontaneous aberrations
Chromatid aberrations	205	17.2 ± 1.1	382	29.6 ± 1.3	198	9.9 ± 0.7
Chromosome aberrations	1	0.1 ± 0.1	45	3.0 ± 0.5	161	4.1 ± 0.5
Total number of aberrations	206	17.3 ± 1.1	427	32.6 ± 1.3	359	14.0 ± 0.8
Number of metaphases examined	1173		1282		1918	
		Controls				
Chromatid aberrations	1	0.2 ± 0.1	1	0.2 ± 0.2	15	0.4 ± 0.1
Chromosome aberrations	0	0	2	0.5 ± 0.3	147	4.3 ± 0.3
Total number of aberrations	1	0.2 ± 0.1	3	0.7 ± 0.4	162	4.7 ± 0.3
Number of metaphases examined	500		402		3442	

Table 3. *Effect of ethylenimine on Crepis capillaris seeds stored at 53 °C*
(Ethylenimine $2.3 \cdot 10^{-2}$ M, treatment period 1 hour)

Treatment	Heating of seeds		Ethylenimine	
Types of chromosome aberrations	No. of aberrations	% after deducting spontaneous aberrations	No. of aberrations	% after deducting aberrations obtained upon heating
Chromatid aberrations	14	0.6 ± 0.15	122	20.8 ± 1.7
Chromosome aberrations	125	4.8 ± 0.4	67	6.8 ± 0.9
Total number of aberrations	139	5.4 ± 0.5	189	27.6 ± 1.9
No. of metaphases examined	2461		569	

the influence of storage on the mutagenic action of alkylating agents is correct, treatment of seedlings with a mixture of ethylenimine and substances from seeds in which only chromatid rearrangements occur spontaneously and after exposure to ethylenimine should not result in chromosome aberrations. And indeed, only chromatid aberrations were recorded (Table 5). When seedlings were treated with a mixture of ethylenimine and substances isolated from seeds stored for 4—7 years, we found chromosome aberrations. Their proportion was about the same (about 5%) as in seeds treated with ethylenimine after storage for 3—5 years (Table 1).

This experiment strongly suggests that the reaction of ethylenimine with substances from seeds stored for long periods leads to the formation of a secondary mutagen showing a different type of action. The formation of such a mutagen may account for the presence of chromosome aberrations when seeds are treated with alkylating compounds.

To conclude, it is worth noting that the interaction between ethylenimine and seed metabolites not only altered the mutagen action qualitatively, but also considerably increased the mutagen activity. This phenomenon will be dealt with in our next communication.

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Table 4. *Effect of ethylenimine (concentration* $4.6 \cdot 10^{-2} M$*; treatment for three hours) on Crepis capillaris seeds with different rates of germination*

Treatment	Chromosome aberrations (%)	Seeds stored for two months after collection. Time of germination and fixation (hours)			Seeds stored for seven months after collection. Time of germination and fixation (hours)		
		24–46	100–150	200–240	30–50	150	215–240
Ethylenimine	Chromatid	52.7 ± 3.0 (148)	43.2 ± 1.7 (383)	12.9 ± 1.5 (65)	45.2 ± 3.0 (130)	39.5 ± 3.2 (90)	15.1 ± 1.6 (76)
	Chromosome	0	1.4 ± 0.4	2.2 ± 0.7	0	5.3 ± 1.4	15.1 ± 1.6
	Total No. of aberrations	52.7 ± 3.0 (148)	44.6 ± 1.7 (395)	15.1 ± 1.6 (76)	45.2 ± 3.0 (130)	44.8 ± 3.2 (102)	30.2 ± 2.0 (152)
	No. of metaphases examined	281	888	502	287	228	504
Controls	Chromatid	0 (0)	0.4 ± 0.2 (4)	0.2 ± 0.2 (1)	0.2 ± 0.1 (1)	0.3 ± 0.3 (1)	0.5 ± 0.3 (3)
	Chromosome	0 (0)	0.4 ± 0.2 (4)	0.4 ± 0.3 (2)	0.2 ± 0.1 (1)	0.3 ± 0.3 (1)	0.4 ± 0.3 (2)
	Total number of aberrations	0 (0)	0.9 ± 0.3 (8)	0.6 ± 0.3 (3)	0.4 ± 0.3 (2)	0.6 ± 0.4 (2)	0.9 ± 0.4 (5)
	No. of metaphases examined	1012	894	533	500	324	554

Table 5. *Interaction between ethylenimine and metabolites from Crepis capillaris seeds*

Treatment	Fixation time	No. of metaphases examined	Metaphases with aberrations		Aberrations per 100 cells		
			No.	%	Chromatid	Chromosome	Total
Metabolites from seeds stored for 2 months + ethylenimine	9	656	178	27.2 ± 1.7	33.8	0	33.8
	15	302	221	73.2 ± 2.5	100.0	0	100.0
	20	282	219	77.8 ± 2.4	148.5	0	148.5
Ethylenimine	9	667	19	2.9 ± 0.7	2.7	0.2	2.9
	15	702	30	4.3 ± 0.8	4.3	0	4.3
	20	668	30	4.5 ± 0.8	4.3	0.2	4.5
Metabolites from seeds stored for 4–7 years + ethylenimine	9	172	6	3.6 ± 1.4	1.3	2.3	3.6
	15	1050	125	11.8 ± 1.0	7.6	4.2	11.8
	20	1189	179	15.0 ± 1.0	9.3	5.7	15.0
Ethylenimine	9	184	5	2.5 ± 1.2	1.5	1.0	2.5
	15	1124	102	8.6 ± 0.8	7.4	1.2	8.6
	20	971	52	5.0 ± 0.7	3.6	1.4	5.0
Metabolites from seeds stored for 2 months + buffer	4	1022	0	0	0	0	0
	9	1430	0	0	0	0	0
	15	685	1	0.1 ± 0.1	0.1	0	0.1
Metabolites from seeds stored for 4–7 years + buffer	4	501	1	0.2 ± 0.2	0	0.2	0.2
	9	700	0	0	0	0	0
	15	800	0	0	0	0	0
Controls (buffer)	4	807	4	0.5 ± 0.2	0.4	0.1	0.5
	9	1058	1	0.1 ± 0.1	0.1	0	0.1
	15	546	1	0.2 ± 0.2	0	0.2	0.2

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